Studies regarding the fertilizing capacity of poultry manure biocomposted by fly larvae (Diptera: Stratiomyidae)

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Abstract. The aim of the research conducted in this paper is to test the possibility of utilizing poultry manure biocomposted by *Hermetia illucens* L. fly larvae as an organic fertilizer. Microclimate conditions necessary for a normal biological cycle of the species were ensured for obtaining the larvae. Flies were obtained from initial pupae, which, after mating, oviposited directly on the laying hen manure substrate, where the larvae hatched and started the substrate processing. Larval processing and pupal migration took place in 37 days, resulting in insect biomass and biocompost. Larval biocomposted manure from laying hens was used for testing its fertilizing ability. Three proportions of soil incorporation for manure were formulated for capsicum growth and development. Three experimental lots were established: M (without organic fertilized soil), L_1 (soil with 10% biocompost) and L_2 (soil with 15% organic fertilizer). The capsicum seeds were initially set to germinate, following with the planting in the growth substrate specific to each established lot. To determine the plant growth evolution, measurements were taken every three days and gross values for statistical processing and comparison were recorded. The experiment results show that the plants from the soil with 10% biocompost inclusion had the best growth in the observed period (21 days). In conclusion, the fly larvae (Diptera) provide an efficient solution in poultry manure management. Their ability to digest and retain nutrients convert poultry manure in larval biomass and into an ideal fertilizer for agricultural lands.
Key Words: fertilizer, biocomposted poultry manure, fly larvae, capsicum.

Introduction. Globally, the human population is experiencing an accelerated growth that leads to increased food demand (Cribb 2010; Dar & Gowda 2013). Over the next 50 years, the world population is estimated to reach more than 9 billion people (DESA 2015) and at the same average annual temperatures are expected to increase due to climate change (Intergovernmental Panel on Climate Change 2007). It is expected that feeding the population will become a challenge that could be solved by increasing agricultural productions by more than 70% by 2050 (Bruinsma 2009; FAO 2017; EUROSTAT 2018). This requirement could be achieved by increasing the profitability and efficiency in food production, with minimal environmental consequences (Van den Berg et al 2013; Uushona 2015).

In the short term, crop plants used in animal feeds or human consumption is expected to have a negative impact on the environment. The use of fertilizers for increasing plant production influences water and air quality, particularly contributing to soil and ecosystem degradation, with direct implications for biodiversity (Tomberlin et al 2015). On the other hand, animal proteins have a biological value superior to those of vegetable origin (Tacon & Metian 2008) and meat production implies a high degree of livestock farm intensification and the production of high manure quantities (EUROSTAT 2018). Globally, agricultural investments in improving land, water and soil nutrients use are necessary in order to counteract these negative effects (Dar & Gowda 2013; Uushona 2015), as well as finding new protein sources (Kobayashi et al 2013). According to Veldkamp et al (2012), insect rearing under controlled conditions might prove to be an advantageous solution for obtaining animal protein alternative sources. Insects convert residual proteins from manure and other organic waste into high-quality protein biomass

(Newton et al 2005a, b; Sanchez-Muros et al 2014), thus contributing to the natural recycling of nutrients, subsequently becoming food for many animal species (Surendra et al 2016). During the bioconversion process, the manure mass is reduced by approximately 50% and the decrease of N and P proportion is also notable (Tomberlin et al 2015; Newton et al 2005; Makkar et al 2014). The resulting material can be used as fertilizer on agricultural lands (Salomone et al 2017). Considerable scientific research is carried out for a better understanding and exploitation activities regarding the management of animal manure and vegetal waste with the help of insect larvae (van Zanten et al 2015).

One of the insect species that is currently being studied is the black soldier fly *Hermetia illucens* (Linnaeus, 1758), which belongs to the Diptera order, Stratiomyidae family (Makkar et al 2014; Park 2016). Originally from the tropical, subtropical and temperate regions of the American continent, the species is currently spread worldwide (Martinez-Sanchez et al 2011). It is of great interest for scientists and farmers due to the larvae's ability to digest a wide range of organic material that is converted in valuable larval biomass (Diener et al 2011; Newton et al 2005) and into an ideal fertilizer for agricultural purposes (Newton et al 2005a, b; Tomberlin et al 2015). The biological capacity of the larvae to convert organic waste (van Huis 2013; Makkar et al 2014; Manurung et al 2016) in compost can be exploited using it as an organic fertilizer in agriculture. Some research highlighted this alternative for obtaining organic fertilizer, whilst trying to demonstrate the positive effect on plant growth by soil administration (Choi et al 2009; Rosmiati et al 2017; Xiao et al 2018).

Black soldier fly larvae bioconversion of manure from laying hens was studied through the experiments carried out in this paper. The fertilizing ability of the resulted biocompost was tested by utilizing it for capsicum (*Capsicum annuum*) growth, in a controlled enviroment. The optimal incorporation ratio of biocompost in soil was researched and established.

Material and Method. The research was conducted in the laboratory of Ecology and Environmental Protection of the Animal Science and Biotechnologies Faculty, UASVM Cluj-Napoca, from October 2017 to March 2018. The experiments were carried out in two stages, as follows:

- poultry manure bioconversion: black soldier fly larvae were used to achieve this goal.The experimental biobase (Figure 1) provided necessary microclimate conditions (22-28°C temperature, 40-60% humidity, 10 hours of light/day) for the biological life cycle of the species. Laying hens manure (LM) from the didactic farm of the Animal Science and Biotechnologies Faculty, UASVM Cluj-Napoca;
- testing the fertilizing ability of poultry manure biocompost. The material resulted by larval digestion of laying hen manure (LMBC) was naturally dried and grinded (Figure 2) in order to be homogenized with soil. The soil was collected from the horticultural area of UASVM Cluj-Napoca. The soil fraction was also dried and grinded to fine powder.

Soil and LMBC were used in varying proportions for capsicum plant growth and development (Table 1). Only soil was used in the preparation of the control lot (M) and two experimental lots were formed by homogeneously incorporating LMBC in soil, in a ratio of 10% (L_1) and 15% (L_2). Fifty (50) g of sample was weighed (Denver Instrument electronic analytical weighing scale) from each formed batch and introduced into special mini-greenhouse plant growing devices, consisting of alveolar trays (50 cells/tray) (Figure 3a). Capsicum seeds were purchased from a specialized commercial unit. Seeds have undergone germination (3-5 days at 15°C) to eliminate those that do not meet planting requirements. Each substrate from the growth cells was seeded with one germinated seed (Figure 3b). The growth devices were placed on a multi-rack shelving, where artificial light was provided (Figure 3c). Air humidity was 60% and a constant temperature of 24°C was maintained. Measurements were taken (gradient in cm) for each plant (Figure 3d) every three days after planting and length development was followed over a period of 21 days.

Raw data has been statistically processed (Office Excel) and the differences test was used between two means (test t, $p < 0.05$).

Figure 1. Providing conditions for black soldier fly larvae rearing on laying hen manure (original).

Figure 2. Composted laying hen manure (after drying and grinding).

Table 1

Experimental lots presentation depending on utilized substrate

Specification	*Soil*		*LMBC*	
	%	*Mass (g)*	*%*	*Mass (g)*
M	100	7.500	-	-
L_1	90	6.750	10	750
L_2	85	6.375	15	1.125

LMBC = compost resulted after larval digestion of laying hen manure; M = 100% Soil; L_1 = soil + 10% LMBC; L_2 = soil+ 15% LMBC.

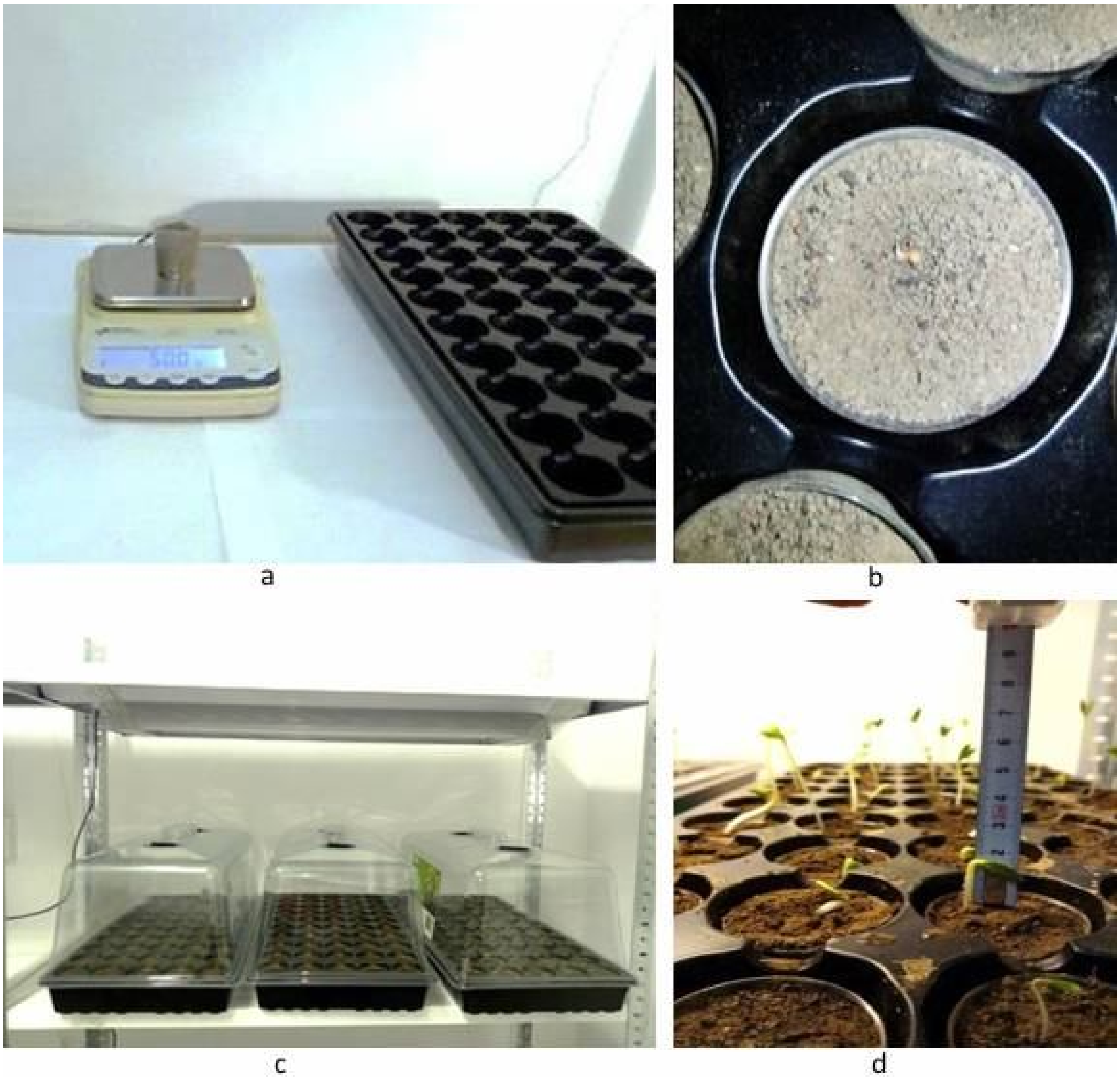

Figure 3. Preparing the experiment for LMBC testing in capsicum development (original).

Results and Discussion. The biological cycle of the reared black soldier fly covered all the development stages. By providing optimal microclimate conditions, adult flies hatched in from initial pupae and mated. The females oviposited directly on the LM substrate. After hatching, the larvae started to feed and the LM substrate was consequently subjected to digestion, thus beginning the bioconversion process. The substrate bioconversion (biocomposting) period is considered to be the larvae growth and development period until the prepupal stage, when it ceases to feed and it migrates (Barros-Cordeiro et al 2014; Oliveira et al 2015).

The larva feeding and migration stage represented, in fact, the period of LM bioconversion in biomass and LMBC. Regarding the *H. illucens* processing period, experiments conducted by other authors showed that it differs depending on the substrate used, but it also depends on environmental parameters (Tomberlin et al 2002; Hardouin & Mahoux 2003; Oliveira et al 2015). Data published by Newton et al (2005a, b) and van Huis (2013) display a 14-day period for swine manure processing, Oonincx et al (2015) and Li et al (2011) obtained a 30-day period for cattle manure, while Dortmans et al (2017) and Xiao et al (2018) indicate a 24-day period for converting poultry manure to organic fertilizer.

In this context, our results regarding *H. illucens* larvae development in laying hen manure (Table 2) point to a period of approximately 37 days for manure bioconversion, resulting in insect biomass and composted organic material.

Table 2

Stages of development in *Hermetia illucens* starting from the pupa stage

Development stage	*No. of days*
Pupae hatching and imago appearance	9
Fly mating and oviposition	7
Larval feeding and prepupae migration	37

The different types of organic waste (cattle or poultry manure, vegetal waste) bioconversion biological ability of the black soldier fly is a topic of interest for many researchers. In experiments, the resulted biocompost was used to replace commercial fertilizers currently applied to different plant cultures. The results showed that plant species sown on soil with different ratios of biocompost presented a better growth rate when compared to the control group (soil and commercial fertilizer). Using the parameters of plant growth rates, the nutrient availability of the biocompost was monitored comparatively to that provided by the commercial fertilizer. The relatively small differences in chemical composition (Choi et al 2009) proved that commercial fertilizer can be successfully replaced with fertilizer obtained by manure larval compost (Rosmiati et al 2017), being also recommended as a sustainable organic fertilizer (Xiao et al 2018).

Regarding the capsicum plants growth under LMBC usage conditions, our results reveal different values of the average plant height, over the 21-day period (Table 3). At the first determination, the control group plants recorded the best growth, but differences ($p < 0.001$) were observed only between M and L_2. At the second measurement it was observed that the best growth mean is correspondent to the L_1 experimental lot plants. Next, each measurement shows differences among the plants from the three lots. Even if no significant differences ($p > 0.05$) exist between the control group and experimental lot L_1, it can be said that higher mean values were obtained by plants grown on soil with 10% biocompost, for all measurements. On the other hand, significant differences ($p < 0.001$) were recorded for all seven measurement sessions between the control group and experimental lot L_2, in favor of the first one. The same statistical differences ($p < 0.001$) occur throughout the monitored period between the two experimental lots, in favor of L_1 plants.

At the end of the studied growth period, the preferences of the capsicum plants seem to be directed to the soil with 10% LMBC, followed by the soil which lacks LMBC. In the case of plants growing on soil with 15% fertilizer, the growth proves to be the least efficient, because the mean values obtained at all measurements were the lowest. It can be argued that the development of capsicum plants according to the applied growth substrate indicates their preference to the soil in which 10% LMBC is incorporated.

Table 3
Capsicum plants mean height values and recorded differences when using different LMBC ratios in soil

Measurement	*Treatment*	*Mean±SD (cm)*	*Statistics*		
			t	*d*	*p*
Day 3	M	1.01±0.1	2.33	0.38	$p < 0.05$
	L_1	0.63±0.12	6.51	0.92	$p < 0.001$
	L_2	0.09±0.09	5.63	0.54	$p < 0.001$
Day 6	M	1.36±0.19	0.19	0.03	$p > 0.05$
	L_1	1.39±0.17	5.98	1.05	$p < 0.001$
	L_2	0.31±0.12	8.91	1.08	$p < 0.001$
Day 9	M	1.62±0.21	0.89	0.19	$p > 0.05$
	L_1	1.81±0.22	5.76	1.17	$p < 0.001$
	L_2	0.45±0.14	9.44	1.36	$p < 0.001$
Day 12	M	1.77±0.25	1.00	0.24	$p > 0.05$
	L_1	2.01±0.24	5.53	1.28	$p < 0.001$
	L_2	0.49±0.16	9.55	1.52	$p < 0.001$
Day 15	M	2.03±0.27	1.00	0.27	$p > 0.05$
	L_1	2.30±0.29	5.95	1.51	$p < 0.001$
	L_2	0.52±0.18	10.04	1.78	$p < 0.001$
Day 18	M	2.07±0.28	1.07	0.31	$p > 0.05$
	L_1	2.38±0.29	5.97	1.58	$p < 0.001$
	L_2	0.49±0.18	10.45	1.89	$p < 0.001$
Day 21	M	2.11±0.29	1.21	0.35	$p > 0.05$
	L_1	2.46±0.31	5.93	1.62	$p < 0.001$
	L_2	0.49±0.18	10.65	1.97	$p < 0.001$

Conclusions. A substrate that can be used as an organic fertilizer can be obtained by larval composting of poultry manure. Another result is the insect biomass, which can be used as an alternative protein source in different farm species feeds (swine, poultry, fish), depending on the harvest stage. It can also be directed towards biodiesel fuel production. Concluding, an accelerated bioconversion of manure from poultry farms can be obtained by using *Hermetia illucens* L. larvae. Both the composting period and the environment negative impacts are notably reduced, another advantage being the partial recovery of undigested protein nitrogen from manure. Following larval digestion, the resulted biocompost can be used as a horticultural and agricultural fertilizer. The use of larval biocomposted laying hen manure as an organic fertilizer requires more tests and analyzes.

References

Barros-Cordeiro K. B., Báo S. N., Pujol-Luz J. R., 2014 Intra-puparial development of the black soldier-fly, *Hermetia illucens*. Journal of Insect Science 14(83).

Bruinsma J., 2009 The resource outlook to 2050. In: FAO Expert Meeting on How to Feed the World in 2050. Rome, Italy, 24-26 June, pp. 1-33.

Choi Y. C., Choi J. Y., Kim J. G., Kim M. S., Kim W. T., Park K. H., Bae S. W., Jeong G. S., 2009 Potential usage of food waste as a natural fertilizer after digestion by *Hermetia illucens* (Diptera: Stratiomyidae). International Journal of Industrial Entomology 19(1):171-174.

Cribb J., 2010 The coming famine: risks and solutions for global food security. Agricultural Science 22(1):24-30.

Dar W. D., Gowda C. L. L., 2013 Declining agricultural productivity and global food security. Journal of Crop Improvement 27(2):242-254.

Diener S., Studt Solano N. M., Roa Gutiérrez F., Zurbrügg C., Tockner K., 2011 Biological treatment of municipal organic waste using black soldier fly larvae. Waste and Biomass Valorization 2(4):357-363.

Dortmans B., Diener S., Verstappen B. M., Zurbrügg C., 2017 Black soldier fly biowaste processing - a step by step guide. Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland, 87 pp.
Hardouin J., Mahoux G., 2003 Zootechnie d'insectes - elevage et utilisation au benefice de l'homme et de certains animaux. Bureau pour l'Echange et la Distribution de l'Information sur le Mini-élevage (BEDIM), 164 pp.
Kobayashi M., Msangi S., Batka M., Vannuccini S., Dey M. M., Anderson J. L., 2015 Fish to 2030: the role and opportunity for aquaculture. Aquaculture Economics and Management 19(3):282-300.
Li Q., Zheng L., Qiu N., Cai H., Tomberlin J. K., Yu Z., 2011 Bioconversion of dairy manure by black soldier fly (Diptera: Stratiomyidae) for biodiesel and sugar production. Waste Management 31(6):1316-1320.
Makkar H. P. S., Tran G., Heuze V., Ankers P., 2014 State-of-the-art on use of insects as animal feed. Animal Feed Science and Technology 197:1-33.
Manurung R., Supriatna A., Esyanthi R. R., Putra R. E., 2016 Bioconversion of rice straw waste by black soldier fly larvae (*Hermetia illucens* L.): optimal feed rate for biomass production. Journal of Entomology and Zoology Studies 4(4):1036-1041.
Martínez-Sánchez A., Magaña C., Saloña M., Rojo S., 2011 First record of *Hermetia illucens* (Diptera: Stratiomyidae) on human corpses in Iberian Peninsula. Forensic Science International 206(1-3):76-78.
Newton L., Sheppard C., Watson D. W., Burtle G., Dove R., 2005a Using the black soldier fly, *Hermetia illucens*, as a value-added tool for the management of swine manure. Report for Mike Williams, Director of the Animal Poultry Waste Management Center, North Carolina State University, Raleigh, NC, 19 pp.
Newton G. L., Sheppard D. C., Watson D. W., Burtle G. J., Dove C. R., Tomberlin J. K., Thelen E. E., 2005b The black soldier fly, *Hermetia illucens*, as a manure management/resource recovery tool. Symposium on the state of the science of Animal Manure and Waste Management, January 5–7, 2005, San Antonio, Texas, USA, 6 pp.
Oliveira F., Doelle K., List R., O'Reilly J. R., 2015 Assessment of Diptera: Stratiomyidae, genus *Hermetia illucens* (L., 1758) using electron microscopy. Journal of Entomology and Zoology Studies 3(5):147-152.
Oonincx D. G. A. B., van Huis A., van Loon J. J. A., 2015 Nutrient utilisation by black soldier flies fed with chicken, pig, or cow manure. Journal of Insects as Food and Feed 1(2):131-139.
Park K., Kim W., Kim E., Kwak K. W., Choi J. Y., Lee S., Song M., Kim S. H., 2016 Oviposition site preference in black soldier fly, *Hermetia illucens* (Diptera: Stratiomyidae), in artificial rearing system. International Journal of Industrial Entomology 33(2):54-58.
Rosmiati M., Nurjanah K. A., Suantika G., Putra R. E., 2017 Application of compost produced by bioconversion of coffee husk by black soldier fly larvae (*Hermetia illucens*) as solid fertilizer to lettuce (*Lactuca sativa* var. *crispa*): impact to growth. Proceeding of International Conference on Green Technology 8(1):38-44.
Salomone R., Saija G., Mondello G., Giannetto A., Fasulo S., Savastano D., 2017 Environmental impact of food waste bioconversion by insects: application of life cycle assessment to process using *Hermetia illucens*. Journal of Cleaner Production 140:890-905.
Sánchez-Muros M. J., Barroso F. G., Manzano-Agugliaro F., 2014 Insect meal as renewable source of food for animal feeding: a review. Journal of Cleaner Production 65:16-27.
Surendra K. C., Olivier R., Tomberlin J. K., Jha R., Khanal S. K., 2016 Bioconversion of organic wastes into biodiesel and animal feed via insect farming. Renewable Energy 98:197-202.
Tacon A. G. J., Metian M., 2008 Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285:146-158.

Tomberlin J. K., Sheppard D. C., Joyce J. A., 2002 Selected life-history traits of black soldier flies (Diptera: Stratiomyidae) reared on three artificial diets. Annals of the Entomological Society of America 95(3):379-386.
Tomberlin J. K., Van Huis A., Benbow M. E., et al., 2015 Protecting the environment through insect farming as a means to produce protein for use as livestock, poultry, and aquaculture feed. Journal of Insects as Food and Feed 1(4):307-309.
Uushona T., 2015 Black soldier fly (*Hermetia illucens*) pre-pupae as a protein source for broiler production. Thesis (MSc Agric), Stellenbosch University.
Van den Berg F., Lyndgaard C. B., Sørensen K. M., Engelsen S. B., 2013 Process analytical technology in the food industry. Trends in Food Science and Technology 31(1):27-35.
Van Huis A., 2013 Potential of insects as food and feed in assuring food security. Annual Review of Entomology 58:563-583.
Van Zanten H. H. E., Mollenhorst H., Oonincx D. G. A. B., Bikker P., Meerburg B. G., de Boer I. J. M., 2015 From environmental nuisance to environmental opportunity: housefly larvae convert waste to livestock feed. Journal of Cleaner Production 102:362-369.
Veldkamp T., van Duinkerken G., van Huis A., Lakemond C. M. M., Ottevanger E., Bosch G., van Boekel T., 2012 Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study. Report 638, Wageningen UR Livestock Research, Lelystad, 48 pp.
Xiao X., Mazza L., Yu Y., Cai M., Zheng L., Tomberlin J. K., Yu J., van Huis A., Yu Z., Fasulo S., Zhang J., 2018 Efficient co-conversion process of chicken manure into protein feed and organic fertilizer by *Hermetia illucens* L. (Diptera: Stratiomyidae) larvae and functional bacteria. Journal of Environmental Management 217:668-676.
*** DESA, 2015 World Population Prospects: 2015 Revision. Available at: http://www.un.org/en/development/desa/population/events/other/10/index.shtml. Accessed: December, 2017.
*** Eurostat regional yearbook 2018 edition. Available at: https://ec.europa.eu/eurostat/documents/3217494/9210140/KS-HA-18-001-EN-N.pdf/655a00cc-6789-4b0c-9d6d-eda24d412188. Accessed: November, 2018.
*** FAO, 2017 The future of food and agriculture. Trends and challenges. Available at: http://www.fao.org/3/a-i6583e.pdf. Accessed: November, 2018.
*** Intergovernmental Panel on Climate Change 2015 Climate Change 2007: The Physical Science Basis. Available at: http://www.slvwd.com/agendas/Full/2007/06-07-07/Item%2010b.pdf. Accessed: November, 2018.

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